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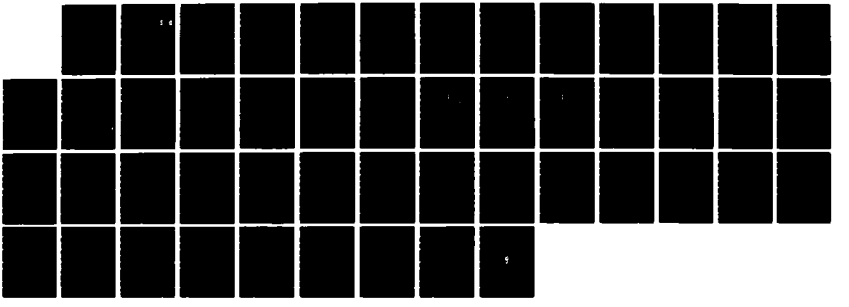

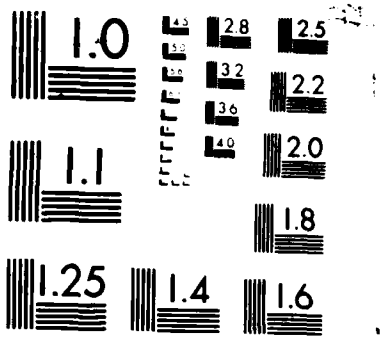

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THROMBOXANE-MEDIATED INJURY FOLLOWING RADIATION

Annual Report

Peter A. Kot, M.D.

August 31, 1985

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The hypothesis under investigation is that moderate levels of radiation exposure result in endothelial and other tissue damage which, in turn, increases in vivo synthesis of thromboxane A2 (TXA2). The observations described in this second annual report indicate that the sources of the radiation-induced increases in TXB2 excretion are diverse, involving organs of both the thorax and upper abdomen. This conclusion is based on the attenuation in the radiation-induced increase in TXB2 excretion seen four hours after 20.0 Gy gamma irradiation with either the thorax or abdomen shielded. An isolated perfused rat kidney model was then developed to determine if the kidneys contribute to the altered cyclooxygenase product release. Urine from the irradiated isolated kidney system showed an elevated excretion of TXB2, PGE2, and 6KPGF1a compared to kidneys from sham irradiated animals. Whole body irradiation of rats with 5.0, 10.0, or 20.0 Gy, also increased pulmonary release of TXB2 four hours after exposure. This is concluded from the

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19. Abstract, continued

isolated perfused rat lung experiments in which lungs from irradiated animals perfused *in vitro* showed significantly elevated TXB2 release at four to 12 hours post irradiation compared to sham irradiated controls. Whole body ionizing radiation exposure also decreased aortic vascular responsiveness to the TXA2 mimic, U46619. Vascular reactivity to phenylephrine was unchanged by radiation exposure. These data show that *in vivo* release of TXB2 involves more than one organ system and as a result, the use of TXB2 as a biological dosimeter requires knowledge as to the organ systems that were irradiated. These data also suggest that regional release of TXB2 varies and as such may provide a means of evaluating regional radiation injury. Finally, the results of the past year show that radiation can alter vascular reactivity to a cyclooxygenase product mimic and that this arachidonate metabolite release is increased following radiation exposure.

The studies projected for next year include 1) a determination of the mechanism(s) by which ionizing radiation alters cyclooxygenase product release, 2) an evaluation of the effect of radioprotectant agents on radiation-induced alterations in vascular reactivity and cyclooxygenase product release, 3) an investigation into the mechanism(s) by which radioprotectants attenuate the radiation-induced increase in cyclooxygenase product release and 4) a series of experiments to determine if analogues of cyclooxygenase products can attenuate radiation injury. The information gained from these proposed studies will add to our understanding of how radiation causes tissue injury. In addition, these proposed investigations will provide insight into a possible bioassay for radioprotectant efficacy.

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Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

BACKGROUND:

Observations on the cellular and tissue effects of ionizing radiation are numerous but the molecular manifestations of radiation injury are poorly understood. Radiation injury is associated with the formation of free radicals and lipid peroxides (1). Several groups of investigators have demonstrated a pivotal role for peroxides in the acceleration of cyclooxygenase activity (2-5). Since cyclooxygenase activity may be one of the rate limiting steps in arachidonate metabolism, and since the cyclooxygenase products have powerful vasoactive and platelet aggregatory properties (6-18), it is possible that these arachidonate metabolites play an important role in radiation-induced vascular injury.

In recognition of this possibility, Eisen and Walker (19) showed that exposure of mice to 7.0 Gy x-irradiation resulted in increased pulmonary and splenic PGE-like activity and increased synthesis of PGF2a. Other studies have shown that hepatic and cerebral PGE-like activity and PGF2a synthesis increased after 5.0 to 7.5 Gy (20). Exposure of mice to 9.0 Gy gamma irradiation resulted in a significantly increased in vitro synthesis of PGE2 and PGF2a by hepatic microsomes and homogenates of brain and testis (21,22). These and other studies demonstrated that in vitro synthesis of the classical products of the cyclooxygenase pathway are increased following exposure to ionizing radiation (19-23).

The effect of ionizing radiation on TXA2 synthesis is less clear. Maclouf et al (24) demonstrated a significant increase in TXB2 release by splenic microsomes isolated from rats exposed to 9.0 Gy whole body irradiation. Steel and Catravas (25) showed increased TXB2 release from guinea pig lung parenchymal strips one to three hours post irradiation. On the other hand, Steel et al (26) failed to show an increase in TXB2 synthesis by guinea pig lung airway tissue following irradiation. Similarly, Allen et al (27) could not demonstrate an alteration in TXB2 release by platelets irradiated in vitro.

During 1984, studies from this laboratory sought to characterize the effect of whole body gamma irradiation on in vivo synthesis of TXB2. These studies showed that urine TXB2 levels were unchanged two to 120 hours following 2.0 Gy ionizing radiation exposure (28,29). Increasing the dose of ionizing radiation to 10.0 Gy significantly ($p < .05$) increased urine TXB2 concentrations four, 12, 24, 72, and 120 hours after exposure. Rats exposed to 20.0 Gy gamma irradiation showed a 233.1% and 105.3% ($p < .05$) increase in urine TXB2 four and 12 hours post irradiation respectively (29). These results were subsequently confirmed by Donlon et al (30) who demonstrated a significant increase in the TXB2 excretion rate 12 and 36 hours after 9.0 Gy whole body irradiation of rats. These studies indicate that in vivo synthesis of TXA2 is increased acutely after whole body irradiation.

In order to determine if this increase in radioimmunoassayable TXB2 was due to an alteration in cyclooxygenase pathway activity, a second series of studies was performed in which rats were pretreated with 5 mg/kg indomethacin or vehicle one hour before exposure to 20.0 Gy. Four hours after irradiation (five hours after indomethacin injection) both irradiated and non-irradiated animals showed a greater than a 90% suppression in urine TXB2 levels compared to their respective vehicle injected groups (29). Thus, the increase in urine TXB2 levels seen acutely after ionizing radiation exposure was a function of altered cyclooxygenase pathway synthesis and/or metabolism (29).

The role of the kidneys in urine TXB2 levels was assessed next. Animals pre-treated with high dose indomethacin had their circulations linked to sham or 20.0 Gy irradiated rats (29). Urine TXB2 levels were significantly higher than pre-cross perfusion levels in the indomethacin-blocked animals indicating the

source(s) of urine TXB2 was/were the circulation of the animals not treated with indomethacin (29). No differences were seen in the urine levels of TXB2 from indomethacin pre-treated rats cross perfused with either control or irradiated animals (29). Thus, urine TXB2 receives a significant contribution from the circulation but the source(s) of the radiation-induced increase could not be determined (29).

Prostaglandins probably play a role in the pathogensis of radiation injury. Recently, Donlon et al. (31) showed that the radioprotectant WR2721 reduced the radiation-induced increase in TXB2, PGE2, and PGF2a excretion. Other studies linking cyclooxygenase product release to tissue injury include the work of Northway et al. (33-35) who demonstrated an attenuation of radiation-induced esophagitis in the opossum by pre-treating animals with indomethacin or aspirin prior to exposure. Animals pre-treated with 16,16 dimethyl prostaglandin E2 before irradiation had augmented esophageal injury compared to untreated controls (33-35). The precise role prostaglandins play in radiation-induced tissue injury remains to be determined.

APPROACH TO THE PROBLEM

Irradiation and Urine Sampling: Previous studies have shown that whole body gamma irradiation at a dose of 20.0 Gy results in a consistent increase in urine TXB2 four hours after exposure. As a result, this dose and time frame was used in most of the studies presented in this report. Male Sprague-Dawley rats (200-250 g) were anesthetized with sodium pentobarbital (30 mg/kg i.p.) before irradiation in order to maintain a consistent pattern of exposure in the radiation chamber. The rats were exposed to either sham irradiation or 20.0 Gy gamma irradiation in a ventro-dorsal orientation to a 7.4×10^{13} becquerel ^{137}Cs radiation source (Best Industries Small Animal Irradiator, Arlington, VA). The rate of delivery was previously calibrated at 0.87 Gy per minute.

Control or irradiated rats were re-anesthetized three hours after exposure and the urinary bladder was exposed through a 1.0 cm midline abdominal incision. The bladder was drained, the urine discarded and the animals left undisturbed for one hour after which time the bladder was again drained and the volume recorded. The urine samples were stored at -20° C prior to the determination of selected cyclooxygenase product concentrations by radioimmunoassay (RIA).

Rats were anesthetized with sodium pentobarbital i.p. and divided into four groups: 1) unshielded, exposed to 15.0 Gy whole body irradiation, 2) abdomen shielded at the level of the kidneys and exposed to 20.0 Gy gamma irradiation, 3) thorax shielded with the same shield used to protect the abdomen and exposed to 20.0 Gy ionizing radiation, and 4) sham irradiated controls. The radiation shield was made from a lead alloy in the shape of a horseshoe. The shield had an internal diameter of 7.0 cm, a width of 4.2 cm, and a thickness of 4.1 cm. The effectiveness of the shield was 92% as determined using lithium iodide thermoluminescent crystals. The unshielded rats were exposed to a 25% smaller dose of radiation because the radiation shield covered 25% of the body. The reduced dose for the unshielded irradiated group meant that organs in this group received 25% less radiation exposure than exposed organs from either of the shielded groups. This approach was chosen to bias the results against ourselves, in that, if a difference was still present in the unshielded group, it would be more likely that the results were real and not an artifact of a higher total body irradiation dose.

In situ Isolated Renal Perfusion: Rats were anesthetized and exposed to 20.0 Gy whole body gamma irradiation or sham irradiation as described above. Three hours later, the animals were re-anesthetized and urine samples removed. These urine samples, obtained prior to the isolation and perfusion of the kidney, were

designated as pre-perfusion urine samples for the isolated perfused kidney studies. After removal of the pre-perfusion urine sample, an endotracheal tube was inserted. The vena cava cephalad to the renal veins and caudad to the diaphragm was isolated and a loose ligature of umbilical tape was placed around the vessel. Three loose ligatures of 4-0 silk were next placed around both the inferior vena cava and the abdominal aorta proximal to the iliolumbar vessels and distal to the renal vessels. The superior mesenteric artery, coeliac artery, and spermatic arteries were then ligated. Finally, a loose ligature of umbilical tape was placed around the abdominal aorta proximal to the renal arteries and distal to the diaphragm. The animal was then anticoagulated with 1000 USP units heparin/kg i.v. (Fig. 1).

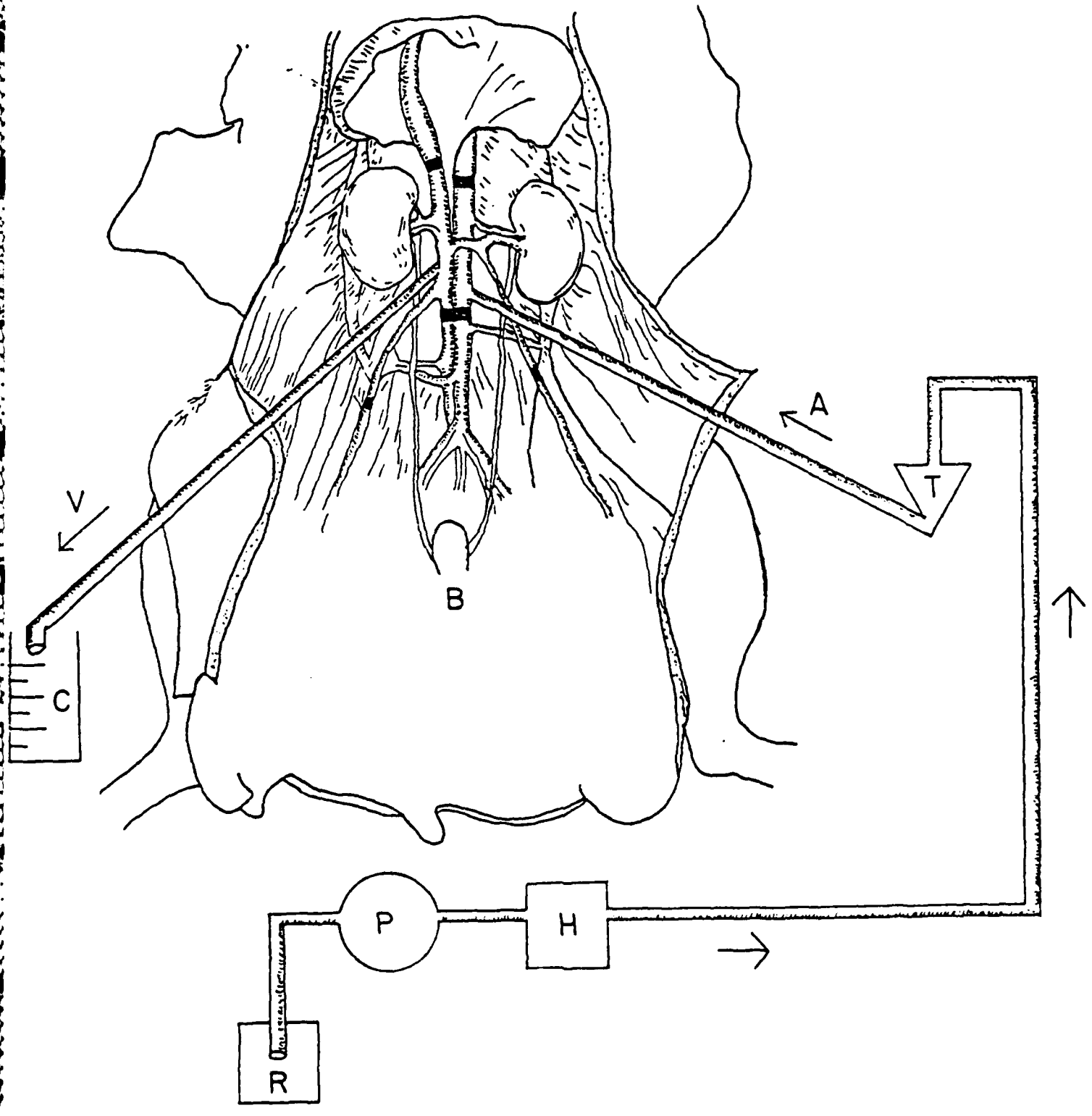
Ten minutes after the adminstration of heparin, the most distal tie around the abdominal aorta and vena cava was tightened. The loose ligature of umbilical tape around the aorta proximal to the renal arteries was next tightened and a catheter (PE-60 tubing) was introduced into the abdominal aorta caudal to the renal arteries and cephalad to the iliolumbar vessels. The renal bed was then cleared of blood by perfusion with Krebs-Ringers bicarbonate (KRB) containing 3% bovine serum albumin and 5.6 mM glucose through the aortic catheter at approximately 5.0 ml/min. The vena cava proximal to the renal veins was then ligated and a second catheter (PE-50 tubing) was placed in the inferior vena cava distal to the renal veins (Fig. 1). The bladder was then drained of urine and the urine was discarded.

The warmed KRB solution (37^{o}) was perfused through the renal bed for 10 minutes at a rate of 3.9 ml/min and a perfusion pressure of 90 mm Hg. Venous effluent samples were taken from the vena caval catheter after five minutes of perfusion. Urine samples were removed from the urinary bladder at the end of the perfusion period and the volume recorded. Aliquots (0.2 ml) of both the venous effluent and urine were removed from each sample and the osmolality and pH determined (Advanced Digimatic Osmometer, Needham, MA) (Fisher Accumet pH Meter Model 310, Pitsburgh, PA). The remaining samples were frozen at -20^{o}C immediately after removal from the animal. Samples were later thawed as a group and assayed for cyclooxgenase products by RIA.

Renal Venous Effluent Extraction: Cyclooxygenase products were extracted from the protein containing venous effluent using the methods of Salmon and Flower (36). Cold acetone (2.0 ml) was added to a 1.0 ml aliquot of venous effluent to precipitate the proteins. The mixture was centrifuged at 1000 x g at 4^{o}C and the supernatant decanted into clean centrifuge tubes. The pellet was washed with an additional 1.0 ml cold acetone, centrifuged, and the supernatant added to that from the first wash. Next, the neutral lipids were removed from the cold acetone by the addition of 2.0 ml hexane. The upper hexane layer, was removed and discarded. The lower acetone layer was acidified to pH 4.0 - 4.5 with citric acid. Chloroform (2.0 ml) was added to the acidified acetone layer and vortexed to extract the prostaglandins from the acetone layer. The upper acetone layer was removed and re-extracted with chloroform. The chloroform layers from both extractions were combined and evaporated to dryness under a vacuum (36). Prior to the determination of cyclooxygenase product concentrations, the extracted prostaglandins were dissolved in RIA buffer back to the original volume.

Lung Perfusion: The animals were re-anesthetized 2, 4, 12, 24, 72, or 120 hours after exposure to 5.0, 10.0 or 20.0 Gy whole body gamma irradiation. The trachea was cannulated and the lungs ventilated with 95% air and 5% CO_2 at a tidal volume of 2.5 ml and a frequency of 55 breaths per minute (Harvard Rodent Respirator, Boston, MA). The blood was removed from the lungs by perfusing them via the catheterized hepatic portal vein with KRB plus 3% bovine serum albumin and 5.6 mM glucose in order to rapidly clear the lungs of blood. Blood was drained from the lungs via the transected abdominal aorta (Fig 2). This route of initial perfusion was chosen because perfusion via the inferior vena cava would put the liver in a

Figure 1: Schematic of isolated perfused rat kidney system. Krebs-Ringers bicarbonate (KRB) plus 3% bovine serum albumin (BSA) and 5.6 mM glucose is perfused from a reservoir (R) and through a pump (P) before being heated to 37° C by a heater (H). Bubbles are removed from the perfusate at bubble trap (T) and then enter the abdominal aorta through the arterial cannula (A). The perfusate drains from the kidney into the inferior vena caval cannula (V) and is collected in a collection vessel (C). Perfused kidney urine is collected directly from the urinary bladder (B).



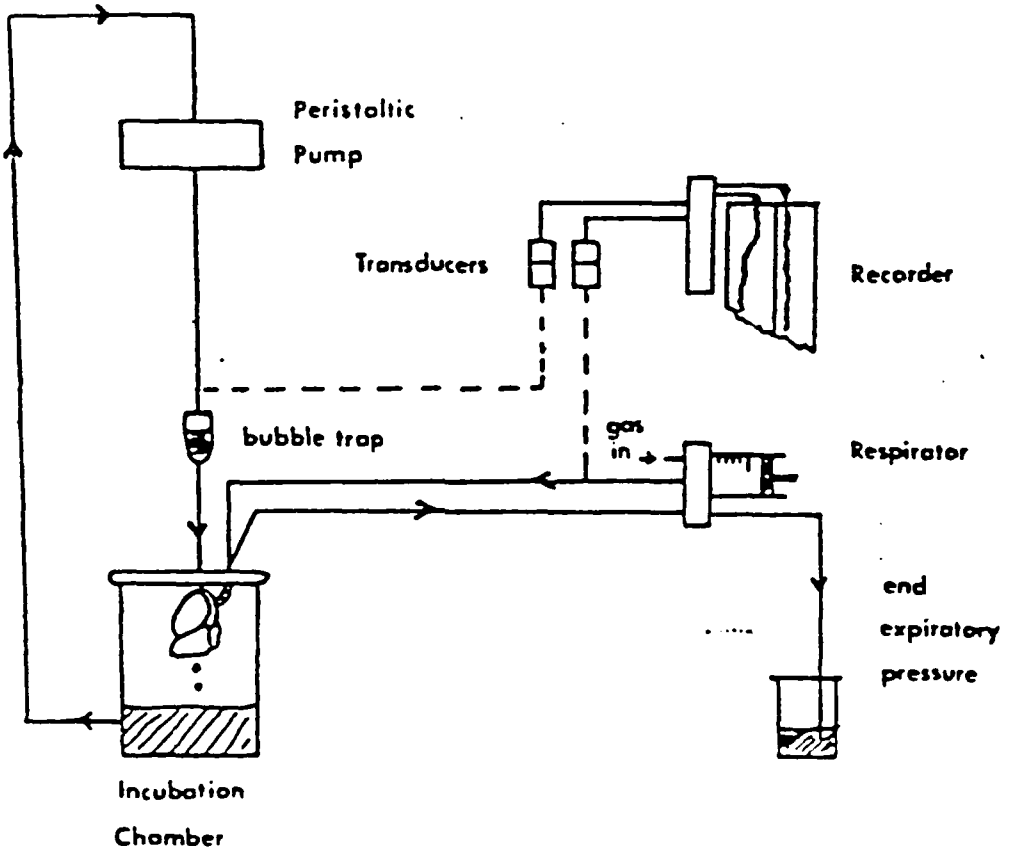


Figure 2. Schematic diagram of lung perfusate system. Rat lungs were ventilated and perfused in vitro. The lungs were ventilated at 2.5 ml per stroke with a pulmonary end expiratory pressure of 1 cm H_2O. The lungs were perfused with Krebs-Ringers bicarbonate plus 3% bovine serum albumin (37° C) at a flow rate of 10 ml/min and a perfusion pressure of 20 mm Hg. The tracheal and perfusion pressures were monitored over the 20 min observation period.

parallel perfusion circuit with the lungs. Since both the lungs and liver are low resistance beds, perfusion via the inferior vena cava would result in poor removal of blood from the lungs and would require a longer period of time for the lungs to clear. By initially perfusing the lungs via the hepatic portal vein, both the liver and lungs were perfused in series so all of the perfusate would ultimately pass through the lungs and the lungs would be more efficiently and completely cleared of blood. This rapid removal of blood would allow for a more rapid transfer of the lungs to the in vitro perfusion chamber and would leave us with a viable pair of lungs.

After the lungs were cleared of blood, the thoracic cavity was opened and the pulmonary artery cannulated. Perfusion of the lungs was transferred from the hepatic portal vein to the pulmonary artery. The lungs were then removed from the animal, suspended in a water-jacketed in vitro perfusion chamber and perfused at a flow rate of 10 ml/min with the temperature maintained at 37°C.

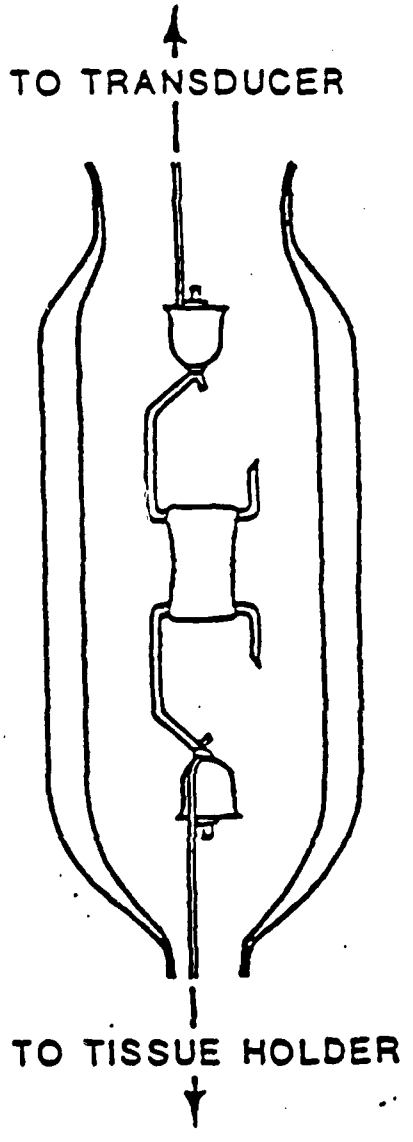
Once the lungs were suspended in the perfusion chamber, the perfusate was allowed to recycle through the lungs. The perfusate entered the lungs through the cannulated pulmonary artery and drained from the lungs via the cut left ventricle. Perfusate samples were taken after 20 minutes of re-circulation, frozen at -20°C, and later thawed as a group for the determination of TXB2 by RIA.

Vascular Reactivity: Rats were anesthetized and exposed to 20 Gy whole body gamma irradiation as previously described. Four hours following irradiation or sham irradiation, the animals were re-anesthetized and a segment of abdominal aorta caudal to the diaphragm and cephalad to the renal arteries was removed. The blood was rinsed from the aortic segment with ice cold KRB. Adherent connective tissue was carefully dissected from the vascular tissue and the section of abdominal aorta was divided into two vascular rings segments two to four millimeters wide.

The isolated ring segments were mounted at one end to a tissue bath and to an isometric tension transducer on the other (Fig. 3). The tissue water-jacketed bath was filled with KRB warmed to 37°C. The pre-load tension was adjusted to 1.0 g. The vascular ring segment was allowed to equilibrate for one hour in this tissue bath. Every 15 minutes during the equilibration period, the pre-load tension was re-adjusted to 1.0 g and the bath fluid exchanged for fresh KRB.

Following the equilibration period, irradiated and sham irradiated vascular tissue rings were exposed to cumulative doses (10^{-9} - 10^{-6} M) of either 9, 11-dideoxy-11a, 9a-epoxymethano-prostaglandin F2a (U46619) or phenylephrine. The vascular reactivity was evaluated by linear regression analysis of a computer generated semilogrithmic plot of the developed isometric tension versus the log of the drug concentration. The correlation coefficient of all curves was greater than 0.90. The slope of the curve, the maximum developed tension, and the ED50 for irradiated and sham irradiated vascular rings exposed to U46619 or phenylephrine were determined from this same curve. The ED50 was calculated based on the semilogrithmic plot. The dose of vascular agonist that induced a maximal recorded response was entered into the computer and the maximal developed tension determined from the semilogrithmic plot. The maximal developed tension was then divided by two to give the 50% maximum developed tension and this number was entered into the computer. The computer, using the 50% maximum developed tension and the semilogrithmic plot of the particular dose-response curve, determined the concentration of agonist necessary to induce a 50% maximum developed tension or ED50.

Radioimmunoassay: Urine TXB2 concentrations were determined by radioimmunoassay using the methods of Granstrom and Kindahl (37,38). An aliquot of urine (20 ul) was added to 300 ul radioimmunoassay buffer (RIA buffer), 80 ul



Rat aortic vessels
(2-3mm ring segments)
3ml organ bath

Krebs Ringer bicarbonate
95% O_2 - 5% CO_2
37°C equilibrated 1 hr

1gm resting tension

Cumulative dose-response curves (isometric)

Figure 3.

Drug Concentrations

U46619	10^{-11} - 10^{-6} molar
Phenylephrine	10^{-9} - 10^{-6} molar

gelatinized radioimmunoassay buffer (gel buffer), 100 ul labelled ligand, and 100 ul antibody. The RIA buffer consists of 1.55 g Trisma 7.0 (Sigma Chemical Co., St. Louis, MO), and 9.0 g NaCl dissolved in 1.0 liter distilled water. To this was added 1.0 ml of 2.0 M $MgSO_4$ and 1.0 ml of 0.2 M $CaCl_2$. Gel buffer consists of RIA buffer to which gelatin was added (0.25 g gelatin per liter RIA buffer). The urine-RIA buffer-Gel buffer-ligand-antibody mixture was incubated overnight at 4° C and the unbound labelled ligand precipitated by the addition of 0.9 ml dextran-coated charcoal (0.3 g Dextran 70 and 3.0 g charcoal suspended in 1.0 liter RIA buffer). The supernatant remaining after centrifugation at 1000 x g was transferred to liquid scintillation vials to which 5.0 ml fluor was added (Atomlight, New England Nuclear, Boston, MA).

The extracted venous effluent samples, after re-constitution to the orginal volume with RIA buffer, were treated in a similar manner as the urine. Since the concentration of arachidonate metabolites was less in the venous effluent than in the urine, larger volumes of the extracted venous effluent were used. For each individual RIA, 100 ul ^3H labelled ligand, 100 ul ligand antibody, and 100 ul gel buffer were mixed together. The venous effluent extract was added in aliquots of 100 ul for the TXB2 and PGE2 assay and in 200 ul aliquots for the 6KPGF1a assay. The remaining volume in each assay tube was either 200 ul or 100 ul RIA buffer such that the final volume in each assay tube was 600 ul. Following incubation, the unbound ^3H labelled ligand was precipitated with dextran-coated charcoal (Sigma Chemical Co., St. Louis, MO) and centrifuged.

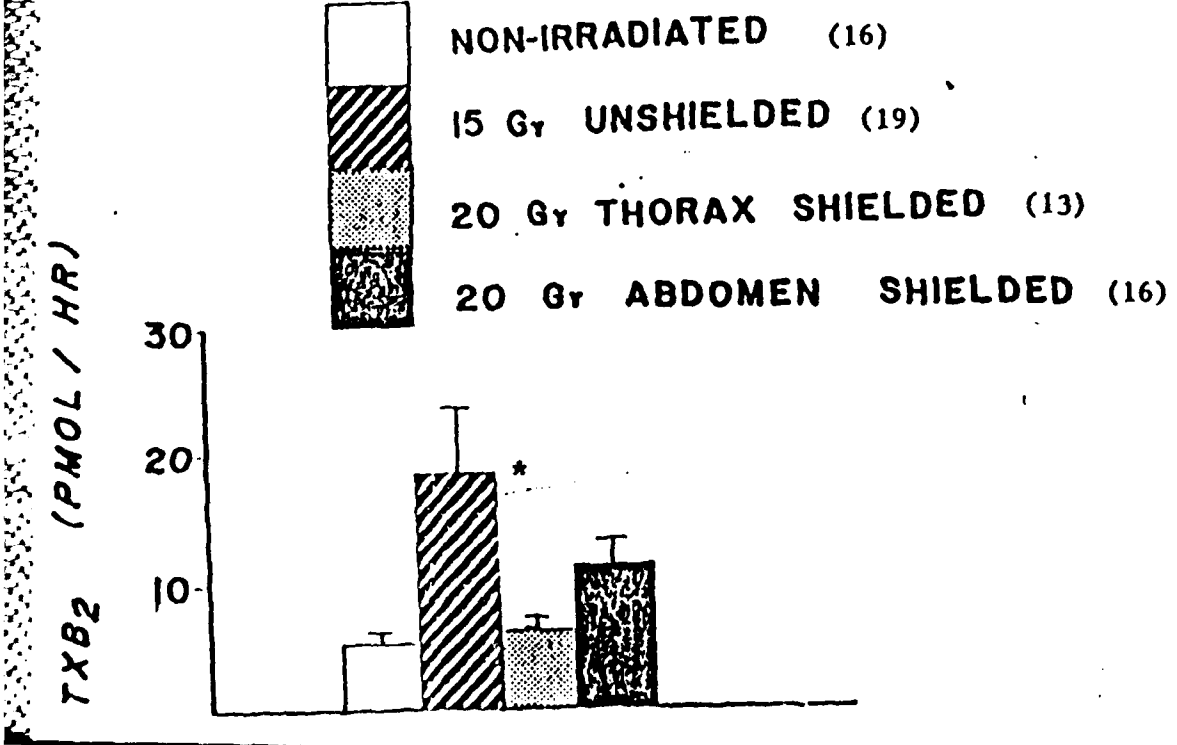
The cross reactivity of the TXB2 antibody was less than 0.3% with PGA2 and less than 0.1% with PGF2a, PGE2 and 6-keto-PGF1a. The TXB2 antibody cross reacted approximately 60% with 2,3 dinor TXB2. Therefore, the TXB2 levels determined represent a mixture of TXB2 and its 2,3 dinor metabolite. The 6-keto-PGF1a antibody showed 10% cross reactivity with PGE2, 7.2% cross reactivity with PGF2a, and less than 1% cross reactivity with either 6-keto-PGE1 or PGF1a. The PGE2 antibody (Accurate Chemical and Scientific Corp., Westbury, NY) cross reacted with PGE1, PGF1a, and PGB1 10%, 3.3%, and 3.0% respectively. Cross reactivity to all other prostaglandins, including 6KPGF1a and TXB2 was less than 1.0%.

The sensitivity of each RIA was evaluated on the basis of the linearity of the standard curves. The minimum detectable values for TXB2, PGE2, and 6KPGF1a were 3.0, 10.0, and 5.0 pg/ml respectively. The maximal detectable levels of TXB2 and PGE2 were 1000 pg/ml and 2500 pg/ml for 6KPGF1a. The volume of urine or extracted venous effluent that was added to the RIA was appropriately adjusted such that the concentrations of the cyclooxygenase products were above the minimum sensitivity but below the maximal detectable levels for each cyclooxygenase product assay.

Results

The urinary TXB2 excretion rate of rats exposed to 15.0 Gy whole body gamma irradiation was 261% ($p < .05$) greater than sham irradiated controls (Fig. 4). Thoracic shielding prevented the radiation-induced increase in TXB2 excretion. Abdominal shielding reduced the radiation-induced increase in the TXB2 excretion rate by 41% but this attenuation was not significantly different from the unshielded irradiated group. A comparison of the abdominal shielding group to controls also showed no significant differences between these two groups (Fig. 4). Thus, thoracic shielding significantly ($p < .05$) reduced TXB2 excretion compared to the unshielded irradiated group but there was no significant difference in the excretion rate when compared to sham irradiated controls. Abdominal shielding partially reduced the radiation-induced increase in TXB2 excretion, but there was no significant difference in the excretion rate of this cyclooxygenase product by abdominal shielded animals compared with either sham irradiated controls or unshielded irradiated rats (Fig. 4). The radiation-induced increase in TXB2 excretion was not

Figure 4: Rats were exposed to sham irradiation, 15.0 Gy whole body irradiation, or 20.0 Gy gamma irradiation with the thorax or abdomen shielded. Four hours following irradiation, urine samples were removed and assayed for TXB2 by RIA. Data are expressed as mean ± SEM. *p <.05 compared to sham irradiated controls.



due to altered rates of urine formation as the urine volume after one hour was not significantly different in any of the groups.

The renal function of perfused kidneys from irradiated and sham irradiated animals was assessed by comparing the urine and venous effluent pH and osmolality. This isolated organ system both acidified and concentrated the perfusate (Table 1). Whole body gamma irradiation did not alter the renal acidifying or concentrating mechanisms nor did it change renal venous effluent pH and osmolality (Table 1).

The urine from both irradiated and sham irradiated perfused kidneys contained significant amounts of TXB2, PGE2, and 6KPGF1a, however, the renal venous effluent contained only TXB2 and 6KPGF1a (Table 2). Comparison of urine and venous effluent TXB2 levels showed no significant difference. Conversely, urine 6KPGF1a concentrations were significantly ($p < .05$) higher than the venous effluent values (Table 2).

The excretion rates of the cyclooxygenase products by intact and isolated perfused kidneys were compared (Table 3). Excretion of TXB2 in sham irradiated perfused kidneys was 641% ($p < .05$) greater than that seen in the intact animal. The TXB2 excretion by kidneys irradiated in vivo and perfused in situ was 262% ($p < .05$) greater than the pre-perfusion excretion rate. Urine PGE2 excretion was not significantly different in the intact organ and the perfused kidney. The pre-perfusion excretion rate of 6KPGF1a was not determined (Table 3).

The effect of ionizing radiation on the renal excretion of cyclooxygenase products was investigated. In the intact animal, whole body gamma irradiation significantly ($p < .05$) increased TXB2 excretion but induced no change in PGE2 release (Table 3). On the other hand, the isolated perfused rat kidney, following irradiation in vivo, demonstrated significant ($p < .05$) increases in TXB2, PGE2, and 6KPGF1a excretion when compared to the rate of release by perfused controls (Table 3).

The effect of exposure to ionizing radiation on the release of cyclooxygenase products into the venous effluent was also assessed (Table 4). Whole body gamma irradiation did not alter the TXB2 or 6KPGF1a release rate. Immunoreactive PGE2 was not detectable in the venous effluent from irradiated or sham irradiated rat kidneys (Table 4).

The regional shielding studies indicated that the lungs may be a site of altered TXB2 synthesis following whole body gamma irradiation. In order to determine if pulmonary TXB2 synthesis was altered by ionizing radiation, rats were exposed to varying doses of gamma irradiation and the lungs isolated and perfused at several time intervals thereafter. Rats exposed to 5.0 Gy whole body gamma irradiation demonstrated a significant increase ($p < .001$) in pulmonary effluent TXB2 concentrations 12 hours after exposure. This same irradiation dose was associated with a significant decrease in TXB2 levels 120 hours post irradiation. The TXB2 values were unchanged at all other observation times following exposure to a dose of 5.0 Gy (Fig 5). Increasing the radiation dose to 10 Gy, resulted in a 157% ($p < .005$) increase in TXB2 pulmonary effluent concentration 12 hours after exposure (Fig. 6). Lung TXB2 effluent concentrations four hours after irradiation with 20 Gy were increased 155% ($p < .001$) but were unchanged two, 12, 24, and 72 hours post exposure (Fig 7).

Previous studies have indicated that the vasculature is very sensitive to radiation injury. In order to assess the role cyclooxygenase products play in radiation-induced vascular injury, studies were performed to determine if radiation exposure altered vascular responsiveness to the TXA2 mimic, U46619. Irradiated vascular rings challenged with U46619 showed a reduced ($p < .05$) vascular

Table 1

Concentrating and acidifying properties of the isolated perfused rat kidney

	Urine pH	Venous Effluent pH	Urine mosm/l	Venous Effluent mosm/l
Sham Irradiated	6.72[a] ±0.06 (13)	7.27[b] ±0.10 (13)	458 ±35 (9)	288[b] ± 2 (9)
20 Gy Irradiated	6.74 ±0.10 (13)	7.38[b] ±0.08 (13)	384 ±21 (10)	290 ± 2 (10)

[a] Data are expressed as mean ± standard error of the mean

[b] $p < .01$ compared to respective urine pH or osmolality

Table 2

Concentrations of selected cyclooxygenase products in the urine and venous effluent of isolated perfused rat kidneys

	TXB2 (pmol)		PGE2 (pmol)		6KPGF1a (pmol)	
	Urine	Venous Effluent	Urine	Venous Effluent	Urine	Venous Effluent
Sham Irradiated	2.98[a] ±0.36 (9)	2.68 ±0.56 (14)	2.72 ±1.32 (8)	ND	3.74 ±1.21 (7)	0.70[c] ±0.19 (10)
20 Gy Irradiated	8.21[b] ±2.18 (11)	3.49 ±0.77 (13)	20.02 ±8.95 (9)	ND	9.79[b] ±1.81 (11)	1.36[c] ±0.73 (6)

[a] Data are expressed as mean ± standard error of the mean

[b] $p < .05$ for irradiated compared to sham irradiated group using the unpaired Student's t-test for 8 - 12 animals per group.

[c] $p < .05$ for venous effluent arachidonate metabolite concentration compared to urine cyclooxygenase product levels analyzed by the paired Student's t-test for 6 - 11 animals per group.

Table 3

Cyclooxygenase product excretion in a one hour period in intact animals and isolated perfused kidney exposed to ionizing radiation

	TXB2 (pmol/hr)		PGE2 (pmol/hr)		6KPGF1a (pmol/hr)	
	Pre-Perfusion	Post-Perfusion	Pre-Perfusion	Post-Perfusion	Pre-Perfusion	Post-Perfusion
Sham Irradiated	0.51[a] ±0.18 (12)	3.78[c] ±0.70 (10)	0.19 ±0.07 (11)	2.83 ±1.21 (8)	N.D.	3.51[c] ±1.08 (6)
20 Gy Irradiated	2.00[b] ±0.76 (10)	7.24 ±1.60 (10)	0.03 ±0.02 (9)	34.60 ±14.62 (9)	N.D.	21.46[b,c] ±5.48 (11)

[a] Data are expressed as mean ± standard error of the mean

[b] $p < .05$ compared to sham irradiated controls by the unpaired student's t-test.

[c] $p < .05$ compared to pre-perfusion excretion rate by the paired Student's t-test

N.D. = Not Done

Table 4

Cyclooxygenase product release in a one hour time period into venous effluent of control and irradiated isolated perfused rat kidneys

	TXB2 (pmol/hr)	PGE2 (pmol/hr)	6KPGF1a (pmol/hr)
Sham Irradiated	84.10[a] (12) ±15.55	ND	20.80 (12) ± 6.95
20 Gy Irradiated	55.19 (11) ±13.58	ND	27.16 (6) ±14.45

[a] Data are expressed as mean ± standard error of the mean

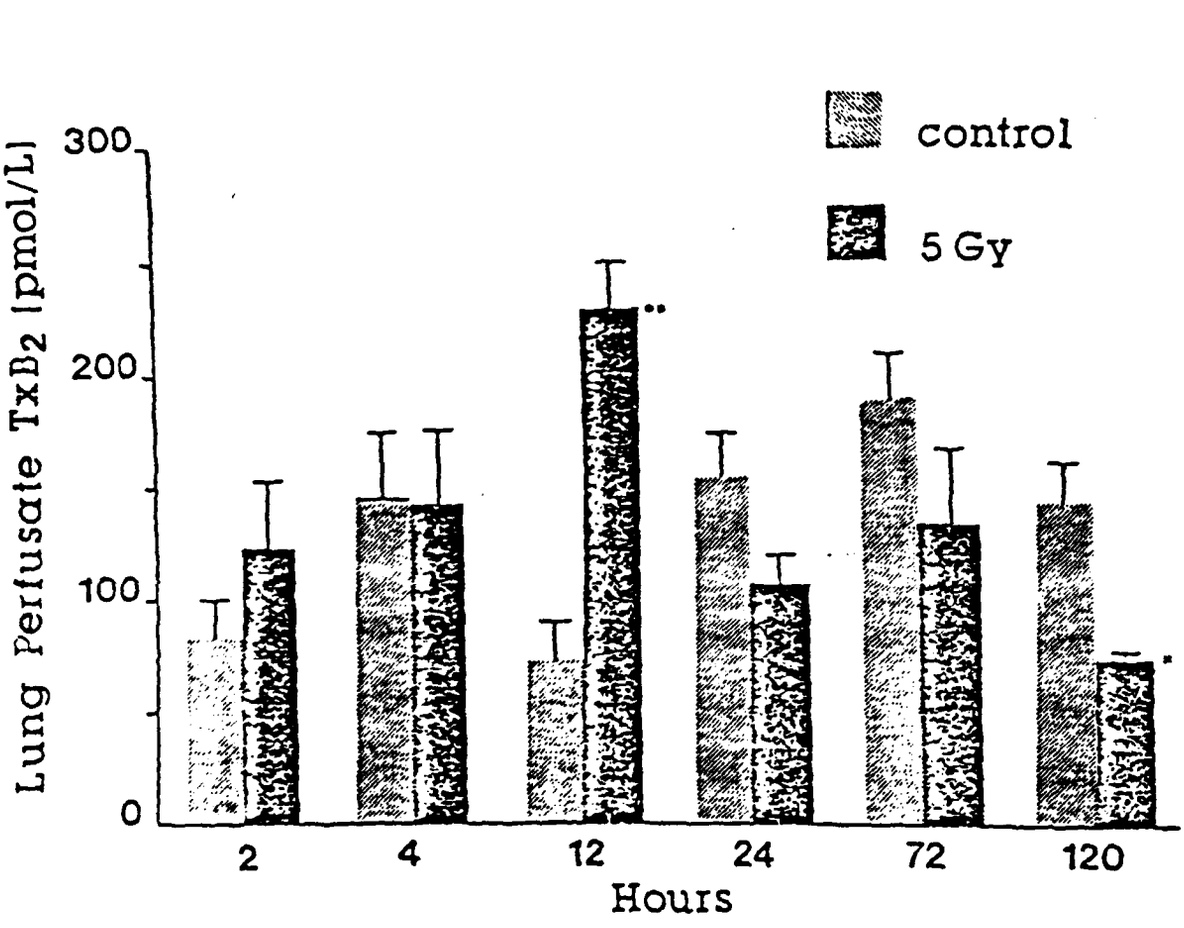


Figure 5. The effect of 5 Gy whole body gamma irradiation on pulmonary synthesis of TXB2. Lung perfusate samples were taken at various times after irradiation and assayed for TXB2 by radioimmunoassay. Data are expressed as mean ± SEM for 6 animals per group. *p<.005, **p<.001

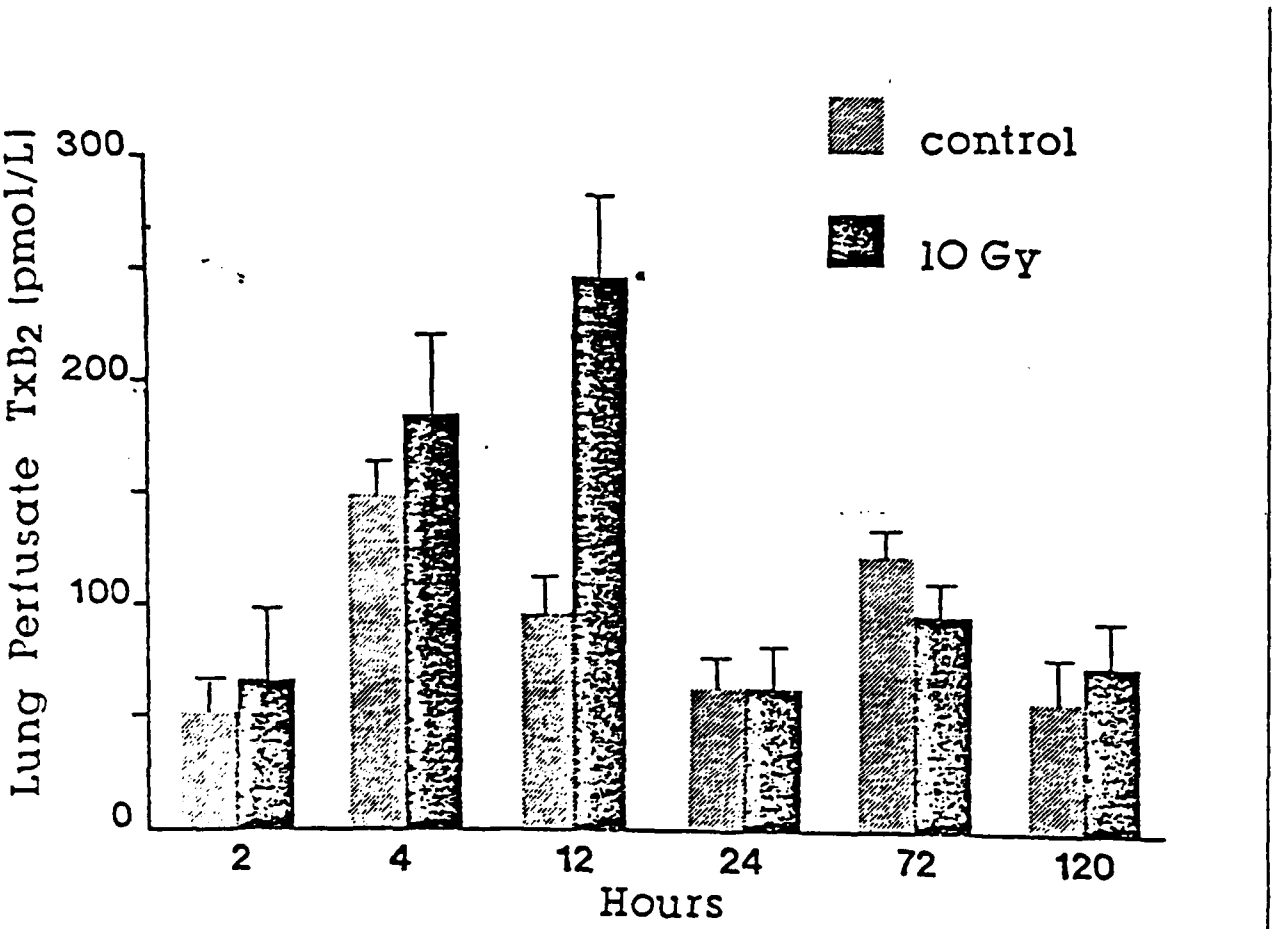


Figure 6. The effect of 10 Gy whole body gamma irradiation on pulmonary synthesis of TXB2. Lung perfusate samples were taken at various times after irradiation and assayed for TXB2 by radioimmunoassay. Data are expressed as mean ± SEM for 6 animals per group. *p<.005

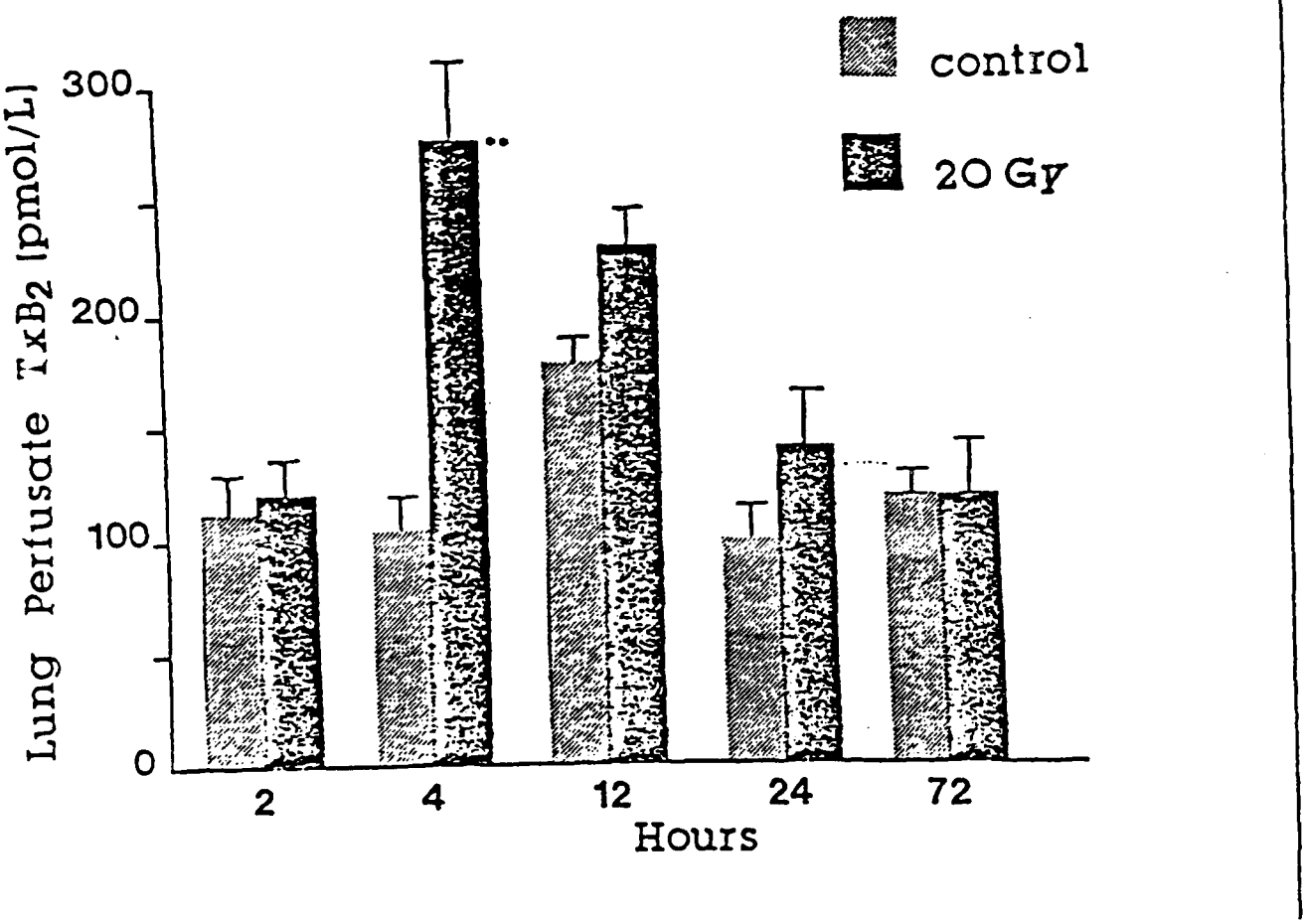


Figure 7. The effect of 20 Gy whole body gamma irradiation on pulmonary synthesis of TXB2. Lung perfusate samples were taken at various times after irradiation and assayed for TXB2 by radioimmunoassay. Data are expressed as mean ± SEM for 6 animals per group. **p<.001

responsiveness at all agonist concentrations tested (Fig. 8). The maximum developed tension was also significantly less ($p < .05$) than sham irradiated vascular tissue (Fig. 8). Rats exposed to 20.0 Gy whole body gamma irradiation four hours prior to the determination of vascular responsiveness to phenylephrine showed no significant difference when compared to non-irradiated controls (Fig. 9). The maximum tension developed in response to phenylephrine was also unchanged from that seen in sham irradiated tissue (Fig. 9).

In order to assess if the altered response of aortic ring segments to U46619 was a receptor phenomenon, the slope of the vascular reactivity curve for the irradiated and sham irradiated vascular segments was determined. Irradiated vascular rings showed a 38.3% ($p < .05$) depression in the slope of the U46619 reactivity curve compared to sham irradiated aortic tissue (Table 5). The slope of the phenylephrine vascular reactivity curves for irradiated and sham irradiated controls was not significantly different (Table 5).

The effect of ionizing radiation exposure on the ED50 of U46619 and phenylephrine was also evaluated. Aortic tissue irradiated in vivo demonstrated no change in the ED50 for either the U46619 or the phenylephrine concentration response curves.

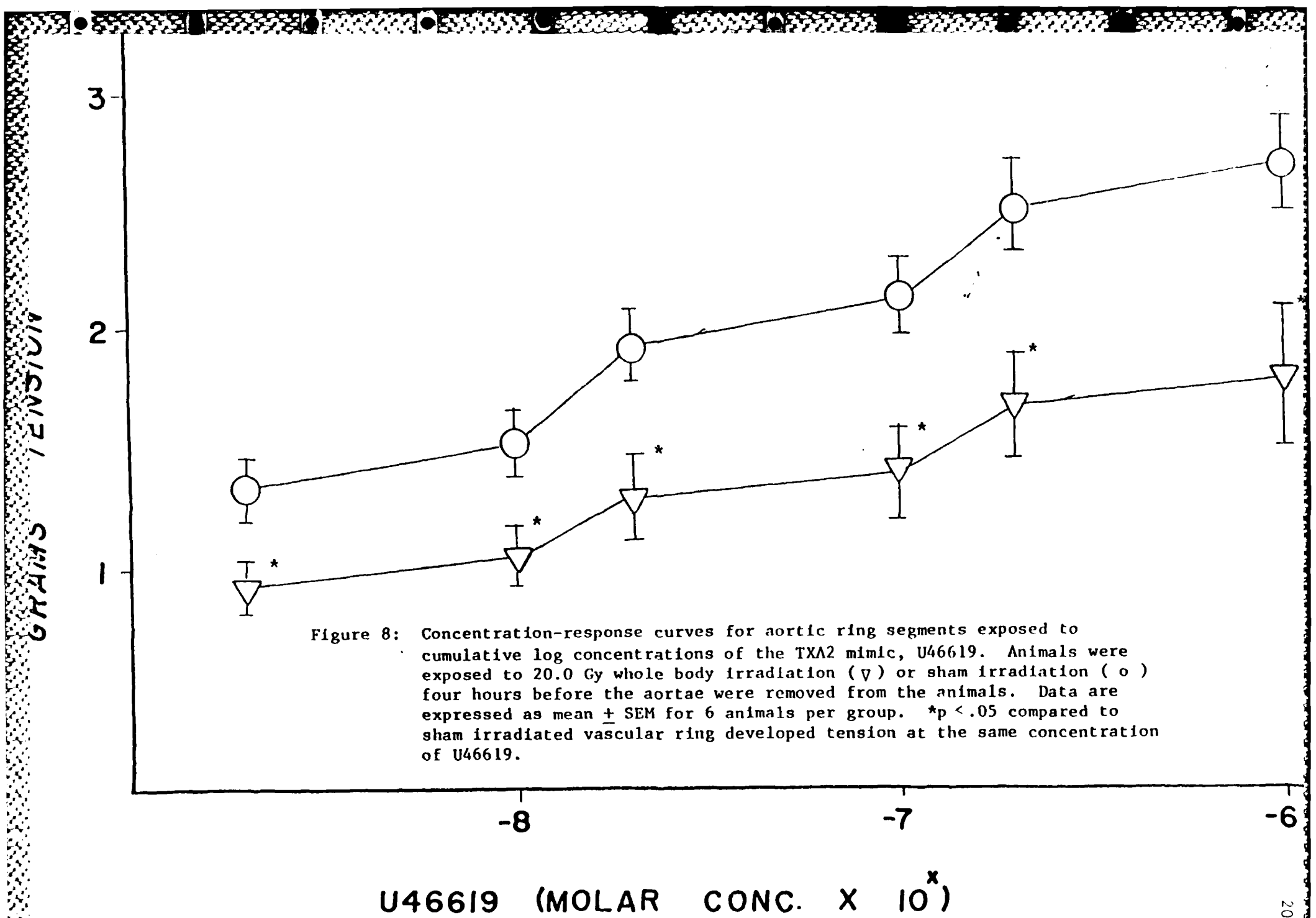


Figure 8: Concentration-response curves for aortic ring segments exposed to cumulative log concentrations of the TXA2 mimic, U46619. Animals were exposed to 20.0 Gy whole body irradiation (▽) or sham irradiation (o) four hours before the aortae were removed from the animals. Data are expressed as mean ± SEM for 6 animals per group. *p < .05 compared to sham irradiated vascular ring developed tension at the same concentration of U46619.

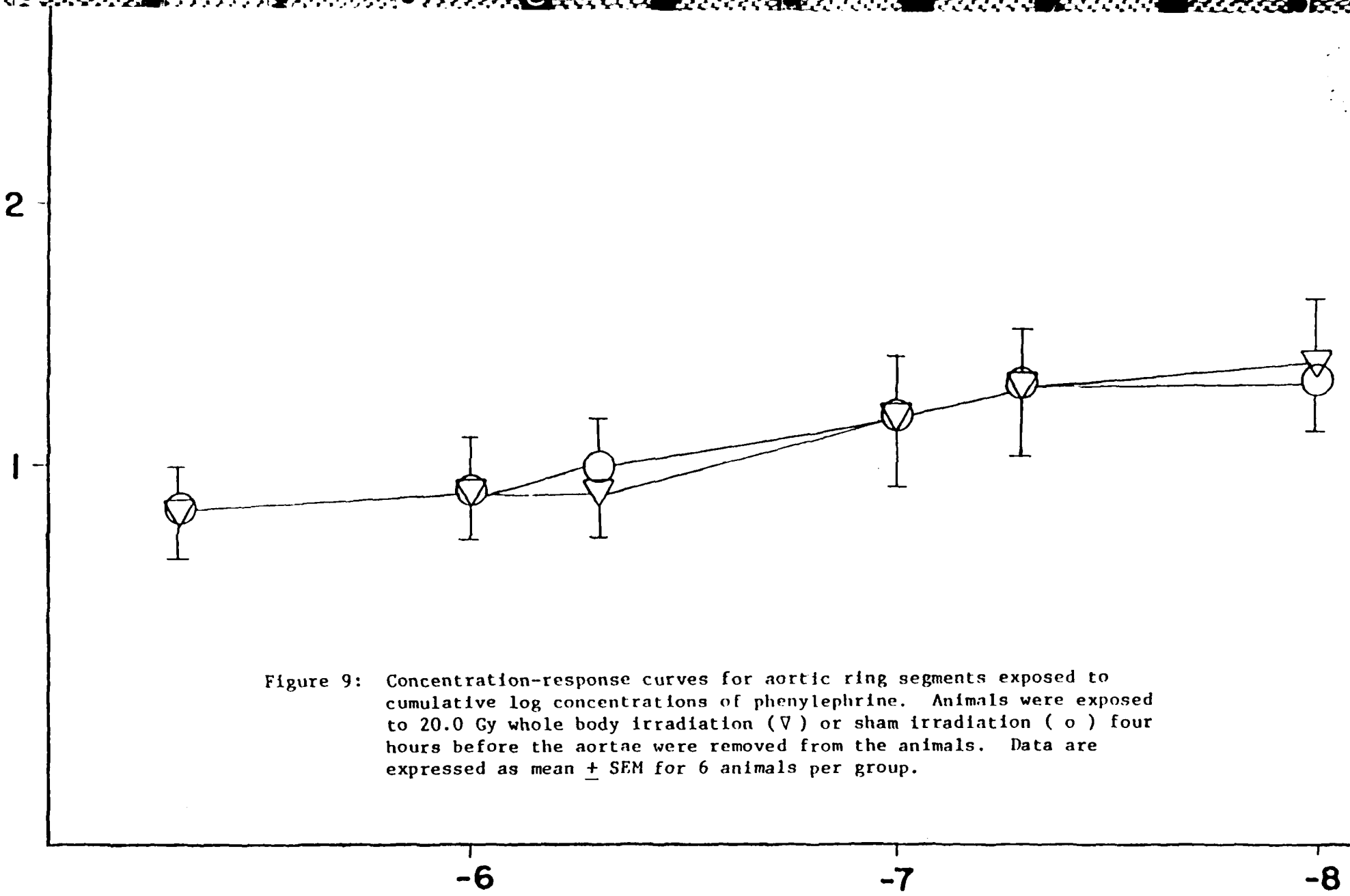


Figure 9: Concentration-response curves for aortic ring segments exposed to cumulative log concentrations of phenylephrine. Animals were exposed to 20.0 Gy whole body irradiation (▽) or sham irradiation (o) four hours before the aortae were removed from the animals. Data are expressed as mean ± SEM for 6 animals per group.

Table 5

Effect of whole body gamma irradiation on abdominal aortic vascular reactivity

	U46619		Phenylephrine	
	Slope	ED50 (10^{-9}M)	Slope	ED50 (10^{-9}M)
Sham Irradiated	0.60[a] ±0.07	17.84 ±4.47	0.23 ±0.02	2.85 ±1.16
20 Gy Irradiated	0.37[b] ±0.08	28.22 ±5.27	0.25 ±0.05	5.88 ±3.57

[a] Data are expressed as mean ± standard error for 6 animals per group

[b] $p < .05$ compared to sham irradiated controls by the unpaired Student's t-test.

Discussion

The regional shielding data presented in this report suggests that the lungs contribute to the radiation-induced increase in TXB2 excretion. This conclusion is supported by the fact that whole body gamma irradiation increases TXB2 release from isolated perfused rat lungs (39). These observations on the altered pulmonary release of TXB2 following irradiation are further supported by the work of Steel et al (25,26) who showed that exposure of guinea pigs to ionizing radiation resulted in increased synthesis of TXB2 by lung parenchymal tissue (25) but not airway tissue (26). The present studies extend previous *in vitro* work from the isolated tissue level to the isolated organ system.

Increases in pulmonary TXB2 synthesis were observed 12 hours after 5.0 and 10.0 Gy and four hours after 20.0 Gy whole body gamma irradiation. While an increase in dose from 10.0 to 20.0 Gy resulted in an earlier alteration in TXB2 release, there was no significant change in the magnitude of the increase. These results differ from previous work presented by this and other laboratories where increasing the exposure level resulted in increased TXB2 excretion (29,31,40). These contradictory responses may be a reflection of the fact that the previous studies evaluated whole body synthesis while the present investigation determined TXB2 synthesis by isolated lungs. The isolated perfused lung data suggests that variations in whole body radiation exposure will alter the temporal release of TXB2 from the pulmonary bed.

Exposure of rats to 5.0 Gy whole body gamma irradiation resulted in a significant decrease in lung perfusate TXB2 concentrations 120 hours post irradiation. These observations are in agreement with the *in vivo* work of Donlon et al (40) who demonstrated a reduction in TXB2 excretion 60 hours after exposure of animals to 1.0 Gy.

Decreased TXB2 excretion was also seen following radiation exposure with the abdomen shielded, but these data had a large variance and failed to achieve significance. Nevertheless, the abdominal organs appear to contribute, at least to some extent, to the increased TXB2 excretion seen following irradiation. With the exception of Maclouf et al (24), little work has been done on radiation-induced alterations in TXB2 release by abdominal organs. In this one study, exposure of mice to 9.0 Gy ^{60}Co radiation, increased TXB2 synthesis by splenic microsomes within 24 hours of exposure. Thus, it is reasonable to conclude that the increased urinary TXB2 excretion observed following whole body irradiation is derived from both thoracic and abdominal organs.

In order to determine the role of the kidneys in this observed response, an isolated perfused rat kidney model was developed and evaluated for functional viability. This was done by comparing the pH and osmolality of the urine formed by the isolated perfused kidney to the pH and osmolality of the venous effluent. This kidney model acidified and concentrated the perfusate. Whole body gamma irradiation did not affect the ability of the isolated perfused kidney to acidify or concentrate urine.

The kidney consists of at least two compartments, both of which can synthesize significant amounts of cyclooxygenase products. The venous effluent, which drains the vascular compartment, contained significant concentrations of TXB2 and 6KPGF1a. The urine, which would receive cyclooxygenase products from both the vascular and renal parenchymal compartments, had measurable quantities of TXB2, PGE2, and 6KPGF1a. The data presented in this report supports the notion that several compartments are involved in the renal synthesis of cyclooxygenase products. This conclusion is based on the observation that the perfused kidney urine contained

significant amounts of PGE2 while the renal venous effluent had undetectable concentrations of this same cyclooxygenase product. Thus, the isolated perfused rat kidney model developed here, offers several advantages over previously described ex vivo techniques in that this rat kidney system can be easily prepared with minimal trauma to the organ. In addition, these data show that this vascularly reactive organ preparation is capable of multi-compartmental synthesis of cyclooxygenase products.

The presence of 6KPGF1a in the venous effluent of isolated perfused kidneys is interpreted as an indication of renal vascular synthesis of prostacyclin (PGI2). Previous studies have shown that PGI2 is a major cyclooxygenase pathway product of vascular tissue (41-43). Unfortunately, other studies have questioned the accuracy of using 6KPGF1a as an indicator of PGI2 synthesis (44,45). Sun et al (45) demonstrated that i.v. infusion of ^{3}H labelled PGI2 results in it's metabolism to several 15-keto metabolites prior to its spontaneous hydrolysis to 6KPGF1a. One of the potential sites for the metabolism of PGI2 to 15-keto products is the kidney. Forstermann and Neufang (46) demonstrated that the kidneys have 15-hydroxyprostaglandin dehydrogenase activity. They also showed that bilateral nephrectomy did not alter plasma concentrations of the 15-keto metabolites of PGI2. Thus, while the kidneys have the enzymatic capacity to degrade PGI2 to 15-keto metabolites, the role they actually play is small. It is therefore concluded, that in the isolated perfused kidney, changes in 6KPGF1a concentrations accurately reflect altered synthesis of PGI2.

The source(s) of vascular effluent TXB2 is/are less clear. On the one hand, TXB2 is the major cyclooxygenase pathway product of platelets (47) and platelets will adhere to damaged vasculature. On the other hand, studies have shown that isolated vascular tissue is also capable of synthesizing TXB2 (41-43). Since the rats, prior to perfusion of the kidneys, are anticoagulated with heparin to prevent activation of the coagulation pathway and aggregation of platelets, and since the kidneys are cleared of blood and perfused by an acellular perfusate, it is unlikely that significant numbers of platelets remain in this organ preparation. If this is the case, then the presence of TXB2 in the venous effluent would represent vascular synthesis of this arachidonate metabolite. More realistically, it is likely that the venous effluent TXB2 concentration is indicative of synthesis by both blood formed elements and vascular tissue. Further studies are necessary to determine the contribution each makes to venous effluent TXB2 concentrations.

In the present study PGE2 could not be detected in the venous effluent from isolated perfused kidneys. These data disagree with Satoh and Satoh (48) who demonstrated that dog renal arteries incubated in vitro for up to 165 minutes released significant amounts of PGE2 in the presence or absence of an intact endothelium. Furthermore, this vascular tissue showed an approximate 80% increase in PGE2 release when challenged with angiotensin II. It may be argued that the lack of detectable PGE2 levels in the venous effluent of the isolated rat kidney may be due to the different species used, however, Gleim et al (49) showed significant amounts of both 6KPGF1a and PGE2 in the renal venous effluent of their isolated perfused rat kidney system. On the other hand, the absence of PGE2 in the renal venous effluent seen in the present study may reflect conversion of PGE2 to other prostaglandins which were not assayed. Credence is lent to this hypothesis by the work of Miller et al (50). In that study, infusion of ^{3}H labelled PGE2 into the isolated perfused rabbit kidney resulted in a rapid conversion of up to 80% of the infused PGE2 to PGF2a. Therefore, it is possible that the absence of assayable PGE2 in the venous effluent of the isolated perfused rat kidney is due to renal metabolism of this arachidonate metabolite. Further studies are necessary to determine if a significant amount of PGE2 metabolism is indeed occurring in the isolated perfused kidney system.

Previous studies from this and other laboratories have shown that whole body gamma irradiation results in an elevated urine TXB2 in intact animals (29-30,40). The present study supports these previous investigations by demonstrating a 292% increase in TXB2 excretion three to four hours after 20 Gy whole body irradiation.

Animals exposed to 20.0 Gy whole body gamma irradiation three to four hours before urine sampling showed no significant differences in PGE2 excretion compared to sham irradiated controls. Previous studies indicate that PGE2 excretion rates are elevated following 9.0 Gy whole body radiation exposure (30,40). This apparent discrepancy may be due to the different urine collection techniques employed in these previous studies (30,40).

The mechanisms for increased release of cyclooxygenase products from intact animals, isolated perfused lungs and isolated perfused kidneys can be divided into three broad possibilities 1) increased arachidonic acid release from cellular membranes, 2) increased activity of the cyclooxygenase pathway enzymes, and 3) decreased metabolism of the cyclooxygenase products once they are synthesized.

Evidence for increased release of arachidonic acid following radiation exposure is found in the work of Hahn et al (51). That study showed that arachidonic acid release from pulmonary endothelial cells exposed to 20.0 Gy irradiation and challenged with bradykinin was 66% greater than the release of this membrane bound fatty acid from sham irradiated cells exposed to the same concentration of this agent (51).

The increased release of TXB2 from intact animals and perfused organs may also be due, in part, to an increased activity of the cyclooxygenase pathway enzymes. This supposition is based on the fact that radiation exposure results in free radical and peroxide formation (1) and this free radical formation may affect cyclooxygenase activity. Seregi et al (4) reported a stimulation of brain cyclooxygenase activity in vitro with the addition of hydrogen peroxide. Hember et al (2) extended these findings by demonstrating a dose-dependent in vitro activation of cyclooxygenase activity by both hydrogen peroxide and lipid peroxides. On the other hand, Egan et al (3) showed that higher concentrations of free radicals inactivated the cyclooxygenase and that this inactivation could be prevented by the addition of free radical scavengers. These studies suggest that radiation-induced free radical and peroxide formation may play a role in the elevated in vivo and in vitro release of cyclooxgyenase products.

Finally, the elevated concentrations of 6KPGF1a and PGE2 in the urine from the isolated perfused kidney may be due to a decrease in the catabolism of this cyclooxygenase product. This proposed mechanism is based on the observations of Eisen and Walker (19) who demonstrated that splenic tissue from 7.0 Gy irradiated mice inactivated PGE1 to a lesser extent than the same tissue taken from sham irradiated controls. Subsequent studies showed that the radiation-induced decrease in 15-hydroxyprostaglandin dehyrogenase activity in splenic tissue was present within four hours of radiation exposure (52). Previous studies have demonstrated the presence of 15-hydroxyprostaglandin dehydrogenase activity in the kidney (46). Other studies have concluded that 15-hydroxyprostaglandin dehydrogenase is a major catabolic enzyme for both PGI2 and PGE2 (44,45,53). Therefore, it is conceivable that the radiation-induced elevation in the excretion of PGE2 and 6KPGF1a by the isolated perfused kidney is also due, in part, to the reduced catabolism of these two cyclooxygenase products.

Previous studies from this laboratory suggested that urine TXB2 excretion may be useful as a biological dosimeter of radiation exposure (29). The data presented in this report do not rule out the use of this cyclooxygenase product for

this purpose. These data suggest that caution is necessary in the interpretation of the radiation response. This conclusion is based on the observation that the increased TXB2 excretion is probably due to altered arachidonate metabolism by pulmonary as well as renal tissue. Therefore, in order to evaluate the dose of radiation exposure using the excretion rate of TXB2, it must first be determined which parts of the body have been exposed.

Whole body ionizing radiation exposure decreased the vascular response of abdominal aortic rings to the TXA2 mimic, U46619 but did not alter the vascular contractile response to phenylephrine. The reduced vascular response to U46619 is in agreement with the work of Narayan and Cliff (54) who showed that local exposure of rabbit ears to 75.0 Gy beta-irradiation resulted in a decrease in microvascular vasomotion four hours after exposure. Conversely, perfusion of rabbit ears 10 to 50 minutes following 80.0 Gy x-irradiation increased the perfusion pressure suggesting that radiation exposure increases vascular tone (55). The data presented in this report, as well as in previous investigations, suggests that ionizing radiation exposure results in either a decreased, increased, or unchanged vascular reactivity depending on the species studied and the vasoactive agonist used. Further studies are necessary to determine the effect of ionizing radiation exposure on vascular responses to various vasoactive agents, and in particular, the cyclooxygenase products.

In summary, whole body ionizing radiation exposure increases TXB2 excretion. The increase in TXB2 excretion appears to be dependent on the dose of whole body radiation exposure. In addition, the radiation-induced increase in urine TXB2 appears to be dependent on regional synthesis, i.e. thoracic or abdominal organs, and may be useful as an indicator of regional exposure as well. The data presented in this report shows that the kidneys and the lungs can, and do contribute to the increased TXB2 excretion following radiation exposure. Finally, the present series of studies characterized a functional deficit in vascular reactivity to a TXA2 mimic which may be associated with the increased in vivo release of this cyclooxygenase product.

Conclusions

The data presented in this second annual report indicate that:

1. The radiation-induced increase in urine TXB2 excretion involes altered release of this cyclooxygenase product by thoracic and abdominal organs.

2. The thoracic organs involved in increased TXB2 excretion include the lungs in that 5.0 to 20.0 Gy whole body exposure resulted in increased pulmonary venous effluent TXB2 concentrations from isolated perfused rat lungs.

3. The abdominal organs involved in increased TXB2 excretion include the kidneys. Whole body ionizing radiation exposure to 20.0 Gy resulted in an increased urine TXB2 excretion rate from isolated perfused rat kidneys.

4. Whole body ionizing radiation exposure resulted in increased synthesis and release of PGE2 and 6KPGF1a from the isolated perfused rat kidney.

5. The acidifying and concentrating mechanisms of isolated perfused rat kidneys were unaffected by exposure to 20.0 Gy whole body gamma irradiation four hours before perfusion.

6. Exposure of animals to 20.0 Gy whole body gamma irradiation decreased vascular responsiveness to the TXA2 mimic, U46619.

7. Rats exposed to 20.0 Gy whole body ionizing radiation showed unchanged vascular reactivity to phenylephrine.

RECOMMENDATIONS

Specific Aims for the Next Year

A. To determine the mechanism(s) of altered cyclooxygenase product release from isolated perfused rat lungs and kidneys following whole body gamma irradiation.

1. Alterations in acyl hydrolase activity will be assessed by the injection of bradykinin, serotonin, and/or histamine into irradiated and sham irradiated isolated perfused rat lungs and kidneys. The rate of cyclooxygenase product release will be determined and compared.

2. Alterations in cyclooxygenase activity will be determined by the injection of exogenous arachidonic acid into irradiated and sham irradiated perfused rat lungs and kidneys. The rate of cyclooxygenase product release will be determined and compared.

3. Alterations in thromboxane synthetase activity will be investigated by the injection of various concentrations of a TXA2 synthetase inhibitor such as OKY-1581, into irradiated and sham irradiated isolated perfused rat lungs and kidneys. The concentrations of OKY-1581 necessary to inhibit TXA2 synthetase from irradiated and sham irradiated organs will be compared.

B. To determine the effect of several radioprotectant compounds on the radiation-induced decrease in vascular contractility to the TXA2 mimic, U46619.

1. The effect of several of the "Walter Reed" compounds on vascular contractility will be characterized as a potential means of evaluating radioprotectant efficacy.

C. To determine the effect of several radioprotectant compounds on the radiation-induced increase in cyclooxygenase product release by isolated perfused rat lungs and kidneys.

1. Doses of available radioprotectants, such as WR2721 and other "Walter Reed" compounds, will be injected prior to, and at varying times after, irradiation. The release of cyclooxygenase products from isolated perfused lungs and kidneys will be determined.

D. To determine the mechanism(s) by which radioprotectants affect the cyclooxygenase product release following ionizing radiation exposure.

1. WR-2721 and other available "Walter Reed" compounds will be infused into irradiated isolated perfused lungs and kidneys and the effect on the cyclooxygenase pathway determined by:

a. evaluating the acyl hydrolase activity by challenging these organs with bradykinin, serotonin, and/or histamine.

b. assessing the cyclooxygenase activity by challenging these organs with exogenous arachidonic acid.

c. investigating the TXA2 synthetase activity by determining the concentrations of OKY-1581 necessary to inhibit TXB2 release from irradiated isolated perfused rat lungs and kidneys pre-treated with the various radioprotectants.

E. To determine the radioprotectant and radiosensitizing effect of prostaglandin analogues.

1. Prostaglandin analogues will be injected before or at varying times after irradiation with an LD50/30 dose of gamma irradiation and the thirty day survival determined.

PROJECTED METHODS

A. To determine the mechanism(s) of altered cyclooxygenase product release from isolated perfused rat lungs and kidneys following whole body gamma irradiation

The purpose of this section is to determine the mechanism(s) of altered cyclooxygenase product release following ionizing radiation exposure. Since previous studies from this laboratory consistently showed that 20.0 Gy whole body ionizing radiation exposure altered cyclooxygenase product release four hours post irradiation, this dose and time frame will be used in all proposed studies.

The methods for irradiaton, *in vitro* pulmonary perfusion, *in situ* renal perfusion, as well as urine and venous effluent sampling have been described in detail in the Approach to the Problem section. In addition, procedures for the extraction of cyclooxygenase products from protein containing venous effluent and radioimmunoassay procedures will be unchanged from those used this past year.

1. Altered acyl hydrolase activity

Male Sprague-Dawley rats (200 - 250 g) will be anesthetized and exposed to 20.0 Gy whole body gamma irradiation or sham irradiation. Three hours later, the animals will be re-anesthetized and the bladder drained of urine. The animals will be left undisturbed for one hour after which time the urine will again be drained, the volume recorded, and the sample frozen at -20°C until they can be assayed by RIA.

The irradiated or sham irradiated kidneys will be perfused with KRB containing 3% bovine serum albumin (BSA) and 5.6 mM glucose at a constant flow rate of 5.0 ml/min. The perfusion pressure will be monitored and once stable, the irradiated or sham irradiated perfused kidneys will be infused with two log unit concentrations of bradykinin, serotonin, histamine, or the appropriate vehicle for 10 minutes. These agents have previously been shown to activate acyl hydrolase activity and to result in increased synthesis of cyclooxygenase products. The concentrations of each of these agents will be comparable to previously measured levels of each compound found following lethal doses of ionizing radiation. The renal venous effluent sample will be taken after 5 minutes of single pass perfusion and the urine samples taken at the end of the perfusion period. The venous effluent and urine samples will be frozen at -20°C until assay by RIA. Should analysis by gas chromatography mass spectrometry (GC-MS) become available, the RIA results will be confirmed by this second assay method.

In a separate series of experiments, rats will be irradiated or sham irradiated as described above. Four hours post irradiation, the animals will be re-anesthetized and the lungs isolated and perfused as previously described. Following an equilibration period of 5 minutes, the lungs will be perfused for 10 minutes with KRB plus 3% BSA and 5.6 mM glucose containing the same two log unit concentrations of bradykinin, serotonin, histamine, or appropriate vehicle used in the renal perfusion studies. The lungs will be perfused for an additional 10 minutes with the perfusate re-cycling through the lungs at a flow rate of 10 ml/min and a perfusion pressure of 15 mm Hg. At the end of the perfusion period, samples from the pulmonary venous effluent will be removed and stored at -20°C. The samples will be assayed as a group by RIA and if available, the pulmonary venous effluent samples will also be assayed by GC-MS.

Interpretation: If irradiated perfused kidneys and/or lungs show increased release of TXB2, PGE2, and 6KPGF1a when challenged with these agents compared to their respective controls, it will be concluded that ionizing radiation exposure increases acyl hydrolase sensitivity to these compounds. Furthermore, these data

would indicate that increased acyl hydrolase activity plays a role in increased cyclooxygenase product release following ionizing radiation exposure. In order to determine if the increase in acyl hydrolase activity is due to the increased circulating concentrations of these compounds, the lungs and/or kidneys from irradiated and sham irradiated animals will be challenged with the calcium ionophore, A23187, and the rate of cyclooxygenase product release determined and compared.

Irradiated perfused organs that show increased cyclooxygenase product release to some but not other challenging compounds that are administered within physiologic concentrations would indicate that some but not other of these agents contribute to increased cyclooxygense product release. These studies would indicate which agents are important in radiation-induced alterations in cyclooxygenase product release and specific blocking agents for those particular compounds will be evaluated for their radioprotectant abilities in a thirty day mortality study.

Isolated perfused organs from irradiated rats that show increased cyclooxygenase product release in response to greater than physiologic concentrations of these challenging agents will be re-assessed with these compounds in combination. This will determine if these agents are acting synergistically on the acyl hydrolases. If this proves to be the case, increased acyl hydrolase activity will be confirmed using A23187.

Should these challenging compounds cause increased cyclooxgyenase product release from one organ system but not the other, it will be concluded that the mechanism of altered cyclooxygenase product release varies depending on the organ system studied. The organ system, lung or kidney, that does not respond to either bradykinin, serotonin, or histamine, will have its cyclooxygenase activity evaluated.

2. Altered cyclooxygenase activity

Male Sprague-Dawley rats will be anesthetized and exposed to 20.0 Gy whole body irradiation or sham irradiation. Four hours later, the kidneys or lungs will be isolated and perfused as previously described. Once the perfusion pressure has stabilized, the isolated organs will be infused with varying concentrations (0.05 to 5 mg/ml) exogenous arachidonic acid for 10 minutes. Renal venous effluent samples taken after 5 minutes of single pass perfusion while the pulmonary venous effluent samples will be collected after 10 minutes of re-cycled perfusion. Urine samples will be taken from the bladder of the isolated perfused kidneys after 10 minutes of single pass perfusion.

Interpretation: If irradiated lungs and/or kidneys infused with exogenous arachidonic acid show significantly higher cyclooxygenase product release than sham irradiated organs infused with the same concentrations of exogenous arachidonic acid, it will be concluded that the altered renal and/or pulmonary release of cyclooxygenase products is due to increased cyclooxygenase activity. Should this response be observed, a concentration-response curve for the cycooxygenase inhibitor, indomethacin, will be generated for the irradiated and sham irradiated organs. The IC50 for each group will then be determined. An increased IC50 for indomethacin from irradiated organs will be taken as further proof of augmented cyclooxygenase activity following whole body ionizing radiation exposure.

Irradiated and sham irradiated perfused organs infused with exogenous arachidonic acid that show similar release rates of cyclooxygenase products would suggest that radiation exposure does not alter cyclooxygenase activity. This response in association with evidence for increased acyl hydrolase activity would indicate that the accelerated release of endogenous arachidonic acid from membrane

phospholipids is responsible for the radiation-induced increase in cyclooxygenase product release. Indications that there is unchanged acyl hydrolase and cyclooxgyenase activity following radiation exposure would suggest that the radiation-induced increase in cyclooxgyenase product release is due to altered synthetase activity after the formation of prostaglandin endoperoxides.

3. Altered thromboxane synthetase activity

The isolated perfused kidneys and lungs will be perfused with KRB plus 3% BSA and 5.6 mM glucose until the perfusion pressure stabilizes. These isolated perfused organs will then be infused with varying concentrations of a TXA2 synthetase inhibitor, such as OKY-1581. The venous effluent samples from kidneys and lungs plus the urine samples from the isolated perfused kidneys will be collected and assayed (RIA and/or GC-MS) for TXB2. From this, a concentration-response curve for OKY-1581 will be generated for both irradiated and sham irradiated organ systems.

Interpretation: Irradiated perfused organs which show a right hand shift in the OKY-1581 concentration-response curve compared to sham irradiated controls, will be taken as evidence of increased TXA2 synthetase activity following ionizing radiation exposure.

A left hand shift in the concentration-response curve of OKY-1581 from irradiated perfused organs compared to sham irradiated controls would indicate that radiation exposure decreases TXA2 synthetase activity. This observation would suggest that the increased release of TXB2 from isolated perfused organs is due to increased substrate availability because of increased acyl hydrolase and/or cyclooxgyenase activity. It is also possible that the increased release of TXB2 in the presence of decreased TXA2 synthetase activity is due to a decreased metabolism of TXA2 once it is formed. This possibility will be investigated should a GC-MS become available.

Concentration-response curves for OKY-1581 in irradiated perfused organs that are similar to that seen in sham irradiated organs would indicate an unchanged TXA2 synthetase activity. This response in conjunction with elevated acyl hydrolase and/or cyclooxygenase activity would suggest that the increase in TXB2 release from perfused organs is due to the increased activity of other enzymes earlier in the cyclooxygenase pathway.

Unchanged concentration-response curves for OKY-1581 in irradiated and sham irradiated perfused organs along with evidence of unchanged acyl hydrolase and cyclooxygenase activity would suggest that there was either a decreased activity of other synthetase enzymes plus shunting of substrate to the TXA2 synthetase or a decreased metabolism of TXA2. Evidence generated to date indicates that most, or all of the synthetase enzymes have unchanged or increased activity as the release of both 6KPGF1a and PGE2 from isolated perfused kidneys is increased following whole body irradiation. Decreased metabolism of TXA2 will be assessed if a GC-MS becomes available.

B. To determine the effect of radioprotectant compounds on radiation-induced alterations in vascular reactivity to U46619

The purpose of this section is to determine if the radiation-induced decrease in vascular reactivity to the TXA2 mimic, U46619 can be prevented by pre-treatment of rats with radioprotectant drugs such as WR2721. The results from this section may be useful in the development of a new, inexpensive, and rapid means of evaluating radioprotectant drugs.

1. Effect of "Walter Reed" compounds on radiation-induced alterations in vascular reactivity

Male Sprague-Dawley rats (200 - 250 g) will be injected with the radioprotectant compound WR2721 or the appropriate vehicle prior to 20.0 Gy whole body gamma irradiation. Four hours later, the animals will be re-anesthetized and the abdominal aorta cephalad to the renal arteries and caudad to the diaphragm will be isolated. The connective tissue will be carefully dissected from the aortic segment and the blood washed from the lumen with ice cold KRB. The aortic segment will be divided into ring segments two to four millimeters long and suspended in a tissue bath as previously described. The tissue bath will be filled with 4.0 ml of KRB and the preload tension will be adjusted to 1.0 g. The vascular ring will be allowed to equilibrate for one hour with the preload tension being reset at 15 minute intervals to 1.0 g. At the same time, the tissue bath will be replaced with fresh KRB.

The irradiated and sham irradiated vascular rings will be challenged with cumulative concentrations of the TXA2 mimic, U46619 and the developed tension determined. The slope, ED50, and maximal developed tension for the irradiated and sham irradiated vascular tissue from animals pre-treated with WR2721 or vehicle will be determined and compared.

Interpretation: Should irradiated vascular rings from animals pretreated with WR2721 have the same U46619 concentration-response curve as sham irradiated vascular tissue, it will be concluded that WR2721 prevented the radiation-induced depression in vascular reactivity. The efficacy of other "Walter Reed" radioprotectants that are available will then be tested in this system in order to establish a data base for the use of this bioassay system for the evaluation of other radioprotectants.

If vascular rings from irradiated, WR2721 pretreated animals show an attenuation of the radiation-induced depression in vascular reactivity to U46619, animals will be treated at several time intervals following radiation exposure with the same compound to determine if these agents must be administered prior to radiation exposure in order to be effective.

Concentration-response curves for U46619 in animals irradiated and pre-treated with WR2721 that are the same as irradiated vehicle treated animals would indicate that the radiation-induced changes in vascular reactivity can not be prevented by pre-treatment of animals with this radioprotectant. It will be concluded that changes in vascular reactivity can not be used as a bioassay of the efficacy of radioprotectant drugs.

Should WR2721 result in a further decrease in the vascular response to the TXA2 mimic, U46619, it will be concluded that WR2721 accentuates the radiation-induce decrease in the contractile response to this agent. The experiments will then be repeated on vascular segments from non-irradiated rats in order to determine if this depression in the vascular contractile response occurs in non-irradiated animals. If this occurs, other radioprotectants will be evaluated for this alteration in vascular reactivity response.

C. To determine the effect of radioprotectants on cyclooxygenase product release from isolated perfused organs

The purpose of this section is to determine if pre-treatment of animals with the radioprotectant, WR2721, will alter the radiation-induced increase in the release of cyclooxgyenase products from isolated perfused rat kidneys and lungs. In

addition, animals will be treated at varying time intervals following radiation exposure to determine if radioprotectants will protect against radiation injury when administered after irradiation. The information gained from this section will be useful in determining the role cyclooxygenase products play in radiation-induced injury. The data will also suggest the time frame necessary for administration of radioprotectants following radiation exposure in order for them to be effective.

Male Sprague-Dawley rats will be anesthetized and injected i.v. with an efficacious dose of WR2721 or other available "Walter Reed" radioprotectant compounds. The animals will be exposed to 20.0 Gy whole body gamma irradiation 30 minutes after the injection of the radioprotectant or the appropriate vehicle. Four hours later, the animals will be re-anesthetized and the lungs and kidneys perfused as previously described. The lungs will be perfused for 10 minutes after the perfusion pressure has stabilized. After the 10 minute perfusion period, samples of pulmonary venous effluent will be removed and stored at -20°C until they can be assayed for TXB2, PGE2, and 6KPGF1a by RIA and/or GC-MS.

Four hours after whole body radiation exposure, the kidneys from radioprotectant or vehicle pretreated irradiated animals will be prepared as previously described. The renal venous effluent will be collected after five minutes of perfusion and the urine from the isolated perfused kidneys will be removed after 10 minutes of single pass perfusion. Both the urine and venous effluent samples will be stored at -20°C until the cyclooxygenase product concentrations can be determined by RIA and/or GC-MS.

Interpretation: If radioprotectant pretreated irradiated organs show a decreased release of cyclooxygense products compared to vehicle pretreated irradiated animals, it will be concluded that the radioprotectant is inhibiting the radiation-induced increase in cyclooxygenase product release. These studies will then be repeated with the radioprotectant being administered at varying time intervals following irradiation in order to determine if these radioprotectants are still efficacious following radiation exposure. Further studies would then be performed on the mechanism(s) by which radioprotectant drugs prevent the radiation-induced increase in cyclooxygenase product release.

Radioprotectant pretreated irradiated organs that show no difference in the rate of cyclooxgyenase product release compared to vehicle pretreated irradiated organs would indicate that these radioprotectants do not attenuate the radiation-induced increase in cyclooxgyenase product release. These results would also suggest that the cyclooxygenase pathway plays a minor role in radiation-induced tissue injury.

Radioprotectant pretreated irradiated organs that show an increased release of cyclooxygenase products compared to vehicle injected irradiated organs would indicate that the radioprotectant is augmenting the radiation-induced increase in cyclooxygenase pathway enzyme activity. These results would suggest that the increase in cyclooxygenase product release seen following lethal doses of whole body ionizing radiation exposure may be a compensatory system. The prostaglandins may be acting as cytoprotectants in this form of injury. If this were the case, the radioprotectants may be preventing tissue injury by increasing the release of these endogenous cytoprotectants.

D. To determine the mechanism(s) of the effect of radioprotectants on the cyclooxygenase pathway

This section is designed to determine how radioprotectants affect cyclooxygenase pathway enzymes. The information gained from this section may be useful in the development of an enzyme assay for radioprotectant drug efficacy studies.

1a. Effect of radioprotectants on acyl hydrolase activity

Male Sprague-Dawley rats will be anesthetized and injected i.v. with an efficacious dose of WR2721 or other available "Walter Reed" radioprotectant compounds. The animals will be exposed to 20.0 Gy whole body gamma irradiation 30 minutes after the injection of the radioprotectant or the appropriate vehicle. Four hours later, the animals will be re-anesthetized and the lungs and kidneys perfused as previously described. The perfusion pressure will be allowed to stabilize before each organ system is infused with either bradykinin, serotonin, histamine, or vehicle at concentrations previously shown to increase cyclooxygenase product release. The effect of WR2721 or other radioprotectants on irradiated acyl hydrolase activity will be evaluated by comparing the cyclooxygenase product release from WR2721 and vehicle pretreated irradiated organs.

Interpretation: If WR2721 attenuates the release of cyclooxygenase products from perfused organs challenged with bradykinin, serotonin, or histamine compared to vehicle pretreated organs challenged with the same concentrations of these agents, it will be concluded that the WR2721 reduces radiation-induced increases in cyclooxygenase product release by modulating the release of endogenous arachidonic acid from membrane phospholipids. The experiments will then be repeated with the calcium ionophore A23187 to determine if the changes in acyl hydrolase activity are due to alterations in membrane calcium permeability.

An attenuation of the radiation-induced increase in cyclooxgyenase product release by WR2721 pretreatment in the absence of reduced acyl hydrolase activity will be interpretated as a reduction in other cyclooxygenase enzyme activity after the release of endogenous arachidonic acid. Studies will then be performed on the effect of WR2721 on cyclooxygenase activity.

Should WR2721 pretreated organs show increased release of cyclooxygenase products that is greater than that seen in vehicle pretreated organs challenged with the aforementioned agents, it will be concluded that WR2721 enhances acyl hydrolase activity. The blunting of the cyclooxygenase product release observed in the previous section would most likely be due to a reduction in enzyme activity after the release of endogenous arachidonic acid from membrane phospholipids.

1b. Effect of radioprotectants on cyclooxygenase activity

Rats will be anesthetized and injected with the same dose of WR2721, other "Walter Reed" radioprotectants, or the appropriate vehicle as used in the previous section. Thirty minutes later, the animals will be irradiated with 20.0 Gy whole body gamma irradiation. Four hours after irradiation, the lungs and kidneys will be isolated and perfused. After the perfusion pressure in each organ system stabilizes, the organs will be infused with varying concentrations of arachidonic acid as previously described. Pulmonary venous effluent samples will be removed from the reservoir after 10 minutes of re-cycled perfusion. Renal venous effluent samples will be taken after 5 minutes of single pass perfusion and urine samples from the isolated perfused kidney will be taken after 10 minutes of perfusion. All samples will be stored at -20°C until they can be assayed by RIA and/or GC-MS.

Interpretation: Venous effluent and urine samples from WR2721 pretreated organs challenged with exogenous arachidonic acid that show reduced release of cyclooxygenase products compared to vehicle pretreated irradiated organs exposed to the same concentrations of arachidonic acid will be taken as an indication that this radioprotectant reduces the radiation-induced increase in cyclooxgyenase activity. These results would suggest that the reduction in radiation-induced release of cyclooxygenase products in the presence of WR2721 is due, in part, to a decreased activity of this enzyme. In order to further confirm these findings, the experiment will be repeated except that the organs will be exposed to increasing concentrations of the cyclooxygenase inhibitor, indomethacin, and the concentration-response curve and IC50 for this drug determined.

Should WR2721 pretreated irradiated organs show an unchanged release of cyclooxygenase products compared to vehicle pretreated irradiated organs, it will be concluded that the radioprotectant does not alter cyclooxygenase activity. These results coupled with the observed decrease in acyl hydrolase activity induced by this radioprotectant would suggest that the decreased release of cyclooxygenase products seen in the previous section in the presence of WR2721 is due to an attenuated acyl hycrolase activity. An unchanged cyclooxgyenase activity in conjunction with an unchanged acyl hydrolase activity would indicate that the decrease in radiation-induced release of cyclooxygenase products from isolated perfused organs is due to a suppression in enzyme activity at the synthetase level or is due to an increase in the metabolism of these arachidonic acid metabolites after they are formed.

WR2721 pretreated irradiated perfused organs that show increased cyclooxgyenase product release when infused with exogenous arachidonic acid compared to vehicle pretreated irradiated organs challenged with the same concentrations of arachidonic acid will be taken as an indication that this radioprotectant increases cyclooxygenase activity. These results along with indications of reduced acyl hydrolase activity would suggest that the attenuation in radiation-induced alterations in cyclooxygenase product release seen in the previous section are due to decreased acyl hydrolase and not due to alterations in cyclooxygenase activity. Should WR2721 increase cyclooxygenase activity and should acyl hydrolase activity also be elevated, the attenuation of radiation-induced cyclooxygenase product release seen in the previous section may be due to decreased synthetase activity. Conversely, the decrease in cyclooxygenase product concentrations may be due to increased metabolism of these cyclooxygenase products once they are formed. In order to test if this is the case, the venous effluent and/or urine will be evaluated for cyclooxygenase metabolites using a GC-MS should it become available.

1c. Effect of radioprotectants on TXA2 synthetase

Rats will be injected i.v. with WR2721, other available "Walter Reed" radioprotectants, or the appropriate vehicle 30 minutes before irradiation with 20.0 Gy whole body ionizing radiation. Four hours after exposure, the kidneys and the lungs will be perfused as previously described. The perfusion pressure will be monitored and after the pressure stabilizes, these organs will be infused with varying concentrations of a TXA2 synthetase inhibitor such as OKY-1581. Venous effluent samples from the lungs will be collected after 10 minutes of single pass perfusion while venous effluent from the kidneys will be collected after 5 minutes of single pass perfusion. Urine samples will be taken after 10 minutes of perfusion. The concentration-response curve for OKY-1581 will be generated and the slope and IC50 determined.

Interpretation: A left hand shift in the OKY-1581 concentration-response curve for irradiated perfused organs from animals pretreated with a radioprotectant will be interpreted as a decrease in TXA2 synthetase activity. This would suggest

that radioprotectants reduce the radiation-induced increase in TXB2 release from isolated perfused kidneys and/or lungs by inhibiting the radiation-induced increase in the TXA2 synthetase enzyme.

Should radioprotectant pretreatment fail to alter the OKY-1581 concentration-response curve compared to vehicle pretreated irradiated rats, it will be concluded that the radioprotectant under investigation does not alter TXA2 synthetase activity. If this occurs, and if this radioprotectant decreases radiation-induced release of TXB2, it will be concluded that this particular agent inhibits either the acyl hydrolase and/or the cyclooxygenase activity. Conversely, if this drug does not alter acyl hydrolase, cyclooxygenase, or TXA2 synthetase activity but if this compound modulates the increased radiation-induced release of TXB2, it will be concluded that the radioprotectant is increasing the metabolism of TXB2. This possibility will be addressed using a GC-MS should the equipment become available.

A right hand shift in the concentration-response curve for OKY-1581 will be taken as an indication of increased TXA2 synthetase activity. Should pretreatment of animals with radioprotectants result in this response, it will be concluded that the radioprotectant increases the TXA2 synthetase activity. The decrease in TXB2 release from isolated perfused organs seen in previous sections following radioprotectant pretreatment would be due to decreased acyl hydrolase activity and/or cyclooxygenase activity. If there is no decrease in these enzyme activities, the decrease in TXB2 levels could be due to decreased metabolism of this arachidonate metabolite. This possibility will be investigated if a GC-MS becomes available.

E. <u>To determine the radioprotectant and radiosensitizing properties of prostaglandin analogues</u>

A mortality study will be performed first by determining a dose of radiation that will result in a 50% mortality of male Sprague-Dawley rats within 30 days following irradiation. Once the radiation dose has been determined, other rats will be injected with varying doses of several prostaglandin analogues such as 16,16 dimethyl prostaglandin E2 or U46619 30 minutes prior to irradiation and the thirty day mortality determined. Controls will be injected with the appropriate vehicle 30 minutes before exposure.

<u>Interpretation:</u> If the injection of the prostaglandin analogue results in a reduced mortality compared to their respective control, it will be concluded that the analogue under investigation is a radioprotectant. This would lend support to the notion that the increased release of prostaglandins is a compensatory mechanism and is acting to reduce tissue injury.

Should the injection of a prostaglandin analogue increase mortality compared to its respective control, it will be concluded that that particular analogue is increasing the sensitivity of the animal to radiation injury. This response would indicate that the suppression of the release of cyclooxygenase products may reduce radiation-induced tissue injury.

An unchanged radiation-induced mortality of prostaglandin analogue pretreated rats would suggest that cyclooxygenase products play little role in mortality. Further studies would be necessary to determine what role, if any, cyclooxygenase products play in the acute tissue injury seen following doses of radiation that cause vascular injury.

STATISTICAL ANALYSIS

In order to analyze the ionizing radiation effect on acyl hydrolase, cyclooxygenase, and TXA2 synthetase, with varying concentrations of the challenging agents and inhibitors, a linear regression analysis of the compound concentration versus cyclooxygenase product release rate will be made. The effect of the challenging compound at any given concentration in irradiated organs compared to sham irradiated organs will be assessed using the unpaired Student's t-test. Similarly, the effect of radioprotectants on the radiation-induced depression in vascular responsiveness to the TXA2 mimic, U46619 will be assessed by generating a semilogrithmic concentration-response curve and analysis of that curve by linear regression analysis. The effect of radioprotectants on the maximal developed tension, ED50, and slope of the concentration-response curve will be evaluated by the unpaired Student's t-test. The effect of radioprotectant pretreatment on the release of cyclooxygenase products from irradiated and sham irradiated perfused kidneys and lungs will be determined using the unpaired Student's t-test. The effect of various radioprotectants on cyclooxygenase pathway enzyme activity will be assessed using linear regression analysis of the concentration of the challenging agents or inhibitors versus the release rate of cyclooxygenase products. The ED50's, slopes, and maximal responses will be determined from these concentration-response curves. The ability of radioprotectants to alter the concentration-response curves will be evaluated by the unpaired Student's t-test. Finally, the effectiveness of prostaglandin analogues as radioprotectants and radiosensitizers will be determined using a 30 day survival study and analyzed using the chi square analysis. For all studies and in all statistical tests used, the confidence interval will be set at 95%.

SIGNIFICANCE

The projected studies for the third year of this contract are logical continuations of the experiments performed during the past two years. The projected studies will:

1. determine the mechanism(s) responsible for the altered cyclooxygenase product release from isolated perfused rat lungs and kidneys following whole body ionizing radiation exposure.

2. determine the effect of various known and putative radioprotectants on the radiation-induced depression in vascular reactivity to the TXA2 mimic, U46619.

3. determine the effect of various known and putative radioprotectants on the radiation-induced increase in cyclooxygenase product release from isolated perfused rat lungs and kidneys.

4. determine the mechanism(s) of the radioprotectant induced attenuation in cyclooxygenase product release from isolated perfused lungs and kidneys following ionizing radiation exposure.

5. determine the radioprotectant and radiosensitizing properties of various prostaglandin analogues.

While the proposed methods differ from those presented in the original contract, the data obtained from these projected studies will do three things: 1) describe the effect of radioprotectants on cyclooxygenase product release from irradiated, isolated perfused organs, 2) describe the effect of radioprotectants on the vascular reactivity changes seen following ionizing radiation exposure, and 3) determine the mechanism(s) by which ionizing radiation exposure increases and radioprotectants decrease cyclooxygense product release.

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